=> d his

(FILE 'HOME' ENTERED AT 19:41:06 ON 11 DEC 2002)

FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 19:41:24 ON 11 DEC 2002

L1 0 S TOCI?(8A)(TETANUS(W)TOXIN OR TT)
L2 350 S TOXIC?(8A)(TETANUS(W)TOXIN OR TT)
L3 284 S TOXIC?(5A)(TETANUS(W)TOXIN OR TT)
L4 194 S TOXIC?(3A)(TETANUS(W)TOXIN OR TT)
L5 3826019 S DOMAIN OR REGION
L6 6 S L4(S)L5
L7 21 S L2(S)L5
L8 8 DUP REM L7 (13 DUPLICATES REMOVED)
L9 8698 S AMINO(W)ACID(5A)(225 OR 245) OR ZINC-BINDING
L10 2 S L2 AND L9
L11 2 DUP REM L10 (0 DUPLICATES REMOVED)

=> d bib ab 1-2 l11

L11 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS
AN 1994:623990 CAPLUS
DN 121:223990
TI Cytotoxic effects of a chimeric protein consisting of tetanus toxin light chain and anthrax toxin lethal factor in non-neuronal cells
AU Arora, Naveen; Williamson, Lura C.; Leppla, Stephen H.; Halpern, Jane L.
CS Lab. Microbial Ecology, NIDR, Bethesda, MD, 20892, USA
SO Journal of Biological Chemistry (1994), 269(42), 26165-71
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB To characterize tetanus toxin effects in non-neuronal cells, a fusion protein consisting of the 254 amino-terminal amino acids of lethal factor (LF) of anthrax toxin and tetanus toxin light chain (LC) was prepd. This protein (LF-LC) inhibited evoked glycine release from primary spinal cord neurons at concns. between 1.0 and 100 ng/mL. LF-LC was cytotoxic to RAW 264.7, ANA-1 cells (mouse macrophage cell lines), and Chinese hamster ovary cells in a dose-dependent manner. These effects required the presence of protective antigen, the receptor binding component of anthrax toxin. In contrast, LF-LC was not cytotoxic to RBL-2H3, Vero, or mouse hybridoma cell lines. Mutagenesis of conserved amino acids (His237 and Glu234) in the **zinc-binding** motif of LC resulted in fusion proteins having no biol. activity. LF-LC did not inhibit regulated secretion of serotonin in RBL-2H3 cells or constitutive secretion in any non-neuronal cell lines as measured in several different assays. The authors suggest that the cytotoxic effects of LF-LC result from inhibition of a specific intracellular membrane fusion event mediated by cellubrevin.

L11 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2002 ACS
AN 1994:127342 CAPLUS
DN 120:127342
TI Functional characterization of the catalytic site of the tetanus toxin light chain using permeabilized adrenal chromaffin cells
AU Hoehne-Zell, Barbara; Stecher, Brigitte; Gratzl, Manfred
CS Abteilung Anatomie und Zellbiologie der Universitaet, Ulm, 89069, Germany
SO FEBS Letters (1993), 336(1), 175-80
CODEN: FEBLAL; ISSN: 0014-5793
DT Journal
LA English
AB The mol. events underlying the inhibition of exocytosis by tetanus toxin were investigated in permeabilized adrenal chromaffin cells. The authors found that replacement of amino acid residues within the putative **zinc binding** domain of the tetanus toxin light chain

such as of histidine (position 233) by cysteine or valine, or of glutamate (position 234) by glutamine completely abolished the effect of the light chains on Ca2+ induced catecholamine release. Dipicolinic acid, a strong chelating agent for zinc, also prevented the effect of the tetanus toxin light chain. Zn2+ and, less potently Cu2+ and Ni2+, but not Cd2+ and Co2+, restored the activity of the neurotoxin. These data show that zinc and the putative **zinc binding** domain constitute the active site of the tetanus toxin light chain. Neither captopril, an inhibitor of synaptobrevin cleavage nor peptides spanning the site of synaptobrevins cleaved by the tetanus toxin in neurons, prevented the inhibition of Ca2+ induced catecholamine release by the tetanus toxin light chain. This suggests that synaptobrevins are not a major target of tetanus toxin in adrenal chromaffin cells.

=>

```
=> d his

     (FILE 'HOME' ENTERED AT 19:41:06 ON 11 DEC 2002)

     FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 19:41:24 ON 11 DEC
     2002
L1             0 S TOCI?(8A)(TETANUS(W)TOXIN OR TT)
L2           350 S TOXIC?(8A)(TETANUS(W)TOXIN OR TT)
L3           284 S TOXIC?(5A)(TETANUS(W)TOXIN OR TT)
L4           194 S TOXIC?(3A)(TETANUS(W)TOXIN OR TT)
L5       3826019 S DOMAIN OR REGION
L6             6 S L4(S)L5
L7            21 S L2(S)L5
L8             8 DUP REM L7 (13 DUPLICATES REMOVED)

=> d bib ab 1-8 l8

L8   ANSWER 1 OF 8  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN   2002:611031  BIOSIS
DN   PREV200200611031
TI   Recombinant toxin fragments.
AU   Shone, Clifford Charles (1); Quinn, Conrad Padraig; Foster, Keith Alan
CS   (1) Wiltshire UK
     ASSIGNEE: Microbiological Research Authority, Salisbury, UK; The Speywood
     Laboratory Limited, London, UK
PI   US 6461617 October 08, 2002
SO   Official Gazette of the United States Patent and Trademark Office Patents,
     (Oct. 8, 2002) Vol. 1263, No. 2, pp. No Pagination.
     http://www.uspto.gov/web/menu/patdata.html. e-file.
     ISSN: 0098-1133.
DT   Patent
LA   English
AB   A polypeptide has first and second domains which enable the
     polypeptide to be translocated into a target cell or which increase the
     solubility of the polypeptide, or both, and further enable the polypeptide
     to cleave one or more vesicle or plasma-membrane associated proteins
     essential to exocytosis. The polypeptide thus combines useful properties
     of a clostridial toxin, such as a botulinum or tetanus
     toxin, without the toxicity associated with the natural
     molecule. The polypeptide can also contain a third domain that
     targets it to a specific cell, rendering the polypeptide useful in
     inhibition of exocytosis in target cells. Fusion proteins comprising the
     polypeptide, nucleic acids encoding the polypeptide and methods of making
     the polypeptide are also provided. Controlled activation of the
     polypeptide is possible and the polypeptide can be incorporated into
     vaccines and toxin assays.

L8   ANSWER 2 OF 8  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN   2001:532742  BIOSIS
DN   PREV200100532742
TI   Retrograde transport of tetanus toxin Fragment C is not blocked by
     vaccination.
AU   Fishman, P. S. (1); Parks, D. A.; Matthews, C. C. (1); Fairweather, N.
CS   (1) Res Service, VA Med Ctr, Baltimore, MD USA
SO   Society for Neuroscience Abstracts, (2001) Vol. 27, No. 1, pp. 1169.
     print.
     Meeting Info.: 31st Annual Meeting of the Society for Neuroscience San
     Diego, California, USA November 10-15, 2001
     ISSN: 0190-5295.
DT   Conference
LA   English
SL   English
AB   The non-toxic binding domain of tetanus
```

toxin (Fragment C or TTC) readily undergoes retrograde axonal transport from an IM injection site even when linked to a variety of other proteins. This property has led to investigation of TTC as a vector to deliver therapeutic proteins to neurons. The vast majority of individuals in the developed world have been vaccinated with tetanus toxoid which prevents clinical tetanus by raising antibodies against the toxin. Circulating antibodies cross react with TTC and may block the delivery of a TTC-linked therapeutic. However, it is uncertain if the immune response is capable of neutralizing an intramuscular depot of protein prior to its internalization by presynaptic nerve terminals where it is inaccessible to antibody. We evaluated uptake of rhodamine labeled TTC following intramuscular injection in 12 normal and 12 vaccinated animals. Vaccination consisted of two injections of tetanus toxoid separated by six weeks. Six weeks after the second vaccination, animals underwent injection of TTC (8ug, 8ul). All animals demonstrated uptake of TTC with fluorescence appropriately localized to the hypoglossal nerve and nucleus. The distribution and intensity of fluorescence within neurons and processes was indistinguishable between the two groups and characteristic of TTC. As expected all vaccinated animals had protective levels of anti-tetanus antibodies as measured by ELISA. We conclude that uptake of TTC by nerve terminals in muscle is an avid and rapid process and is not blocked by vaccination associated with protection from tetanus toxin.

L8 ANSWER 3 OF 8 MEDLINE DUPLICATE 1
AN 2001694063 MEDLINE
DN 21606006 PubMed ID: 11738759
TI Tetanus toxin fragment C-specific priming by intranasal infection with recombinant Bordetella pertussis.
AU Reveneau N; Alonso S; Jacob-Dubuisson F; Mercenier A; Locht C
CS INSERM U447, Institut Pasteur de Lille, 1, Rue du Pr. Calmette, F-59019 Lille, France.
SO VACCINE, (2001 Dec 12) 20 (5-6) 926-33.
Journal code: 8406899. ISSN: 0264-410X.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200204
ED Entered STN: 20011217
Last Updated on STN: 20020413
Entered Medline: 20020412
AB As an alternative to parenteral administration, mucosal administration offers several advantages including the ease of administration, safety and the ability to induce mucosal immunity. As a first step towards nasal administration of important childhood vaccines, we have previously developed attenuated Bordetella pertussis strains able to protect mice against pertussis upon nasal vaccination. Since pertussis vaccines are generally combined with tetanus and diphtheria vaccines, we constructed recombinant B. pertussis strains producing the non-**toxic** protective **tetanus toxin** fragment C (TTFC). TTFC was genetically fused to the N-terminal **domain** of the B. pertussis filamentous haemagglutinin. The hybrid gene was introduced into B. pertussis both on a multi-copy replicative plasmid and as a single copy inserted into the chromosome of a pertussis toxin-producing strain and a toxin-deficient attenuated strain. The hybrid protein was secreted by the recombinant strains. However, the recombinant multi-copy plasmid was unstable in vivo, and immunisation could only be carried out with the strains containing the single-copy chromosomal integration. Both the toxin-producing and the toxin-deficient recombinant B. pertussis strains were able to prime mice for the production of anti-TTFC serum antibodies upon intranasal administration, suggesting the feasibility of using recombinant attenuated B. pertussis for the development of combined childhood vaccines.

L8 ANSWER 4 OF 8 MEDLINE DUPLICATE 2
AN 2001113420 MEDLINE
DN 21002690 PubMed ID: 11126515
TI Interaction of tetanus toxin derived hybrid proteins with neuronal cells.
AU Figueiredo D M; Matthews C C; Parks D A; Fairweather N F; Dougan G; Wilt S G; Fishman P S
CS Neurology Service and, Department of Neurology, Baltimore VAMC, University of Maryland School of Medicine, Baltimore, MD 21201, USA.
NC 1-PO1-AG12992-01 (NIA)
SO JOURNAL OF NATURAL TOXINS, (2000 Nov) 9 (4) 363-79.
Journal code: 9208016. ISSN: 1058-8108.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200102
ED Entered STN: 20010322
Last Updated on STN: 20021026
Entered Medline: 20010215
AB The non-**toxic** ganglioside binding **domain** of **tetanus toxin** (Hc fragment C or TTC) has been studied as a vector for delivering therapeutic proteins to neurons. There is little information on the cellular processing of proteins delivered by linkage to TTC. We have evaluated the cellular handling of a multi-**domain** hybrid protein containing TTC and both the human enzyme superoxide dismutase and the maltose binding protein from E. coli. Binding, internalization, and cleavage of this protein during prolonged incubation with fetal cortical neurons or cells of the N18-RE-105 line was evaluated by immunoblot analysis, ELISA, and immunocytochemistry. Hybrid proteins were bound and internalized in a manner very similar to TTC. Internalized proteins showed long-term stability within cells, and were degraded into predictable large protein fragments in both cell types. Fragments that were cleaved away from the TTC **domain** were released into extracellular fluid after internalization. Proteins coupled to TTC share its long-term stability after cellular internalization. After internalization, dissociation of proteins linked to TTC facilitates their release from the cell, but not into other cellular compartments such as the cytosol. TTC linked proteins are probably enclosed within a stable endosomal compartment throughout their cellular lifetime.

L8 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2002 ACS
AN 1999:388086 CAPLUS
DN 131:43576
TI Vaccines containing attenuated bacteria
IN Chatfield, Steven Neville; Sydenham, Mark; Dougan, Gordon
PA Medeva Europe Limited, UK
SO PCT Int. Appl., 53 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9929342	A1	19990617	WO 1998-GB3680	19981210
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2313703	AA	19990617	CA 1998-2313703	19981210

AU 9914960 A1 19990628 AU 1999-14960 19981210
AU 739191 B2 20011004
EP 1037664 A1 20000927 EP 1998-959023 19981210
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO
JP 2001525375 T2 20011211 JP 2000-524011 19981210
PRAI GB 1997-26233 A 19971211
WO 1998-GB3680 W 19981210

AB The invention relates to a vaccine comprising a bacterium attenuated by a non-reverting mutation in a gene, e.g. surA gene and gene for parvulin (peptidyl-prolyl cis-trans isomerase), encoding a protein which promotes folding of extracytoplasmic proteins. Such mutations were initially identified as being useful in vaccines from a bank of randomly inserted, transposon mutants in which attenuation was detd. as a redn. in virulence of the organism in the mouse model of infection. Site directed mutation of the gene results in a strain which shows at least 4 logs of attenuation when delivered both orally and i.v. Animals vaccinated with such a strain are protected against subsequent challenge with the parent wild type strain. Finally, heterologous antigens such as the non-**toxic** and protective, binding **domain** from **tetanus toxin**, fragment C, can be delivered via the mucosal immune system using such strains of bacteria. This results in the induction of a fully protective immune response to subsequent challenge with native tetanus toxin.

RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 6 OF 8 MEDLINE DUPLICATE 3
AN 1999011483 MEDLINE
DN 99011483 PubMed ID: 9795391
TI Identifying the principal protective antigenic determinants of type A botulinum neurotoxin.
AU Bavari S; Pless D D; Torres E R; Lebeda F J; Olson M A
CS Department of Cell Biology and Biochemistry, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD 21702-5011, USA.
SO VACCINE, (1998 Nov) 16 (19) 1850-6.
Journal code: 8406899. ISSN: 0264-410X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199901
ED Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19990106

AB The neurotoxins from Clostridium botulinum (BoNT serotypes A-G) exert their lethal effect by preventing the release of acetylcholine at the neuromuscular junction. As with **tetanus toxin**, immunization with a non-**toxic** fragment, the 50 kDa C-terminal portion of BoNT/A (Hc; residues 861-1296), protects mice against lethal challenges with the intact toxin. To locate the neutralizing epitopes, several protective monoclonal antibodies (mAbs) against BoNT/A-Hc were isolated and cloned. Specific binding of the mAbs to BoNT/A-Hc was demonstrated by surface plasmon resonance, with Kas in the range of 10(-10) to 10(-11) M. These antibodies recognized a genetically engineered polypeptide (1150-1289) that was previously shown to induce protective immunity. Prior to the determination of the X-ray crystal structure of the tetanus neurotoxin Hc fragment, molecular modelling studies indicated that it contained two highly solvent-exposed loops. Based on these predictions, two 25-mer Hc-peptides corresponding to these two **regions** were synthesized and were demonstrated to bind the neutralizing mAbs. Mice immunized with the Hc-peptides had high levels of antibodies that recognized BoNT/A-Hc. However, immunizations with only one of the Hc peptides protected when mice were challenged with BoNT/A. On the basis of

these analyses, it should be possible to develop small peptides that could be useful in the design of future vaccines against these neurotoxins.

L8 ANSWER 7 OF 8 MEDLINE DUPLICATE 4
AN 97378928 MEDLINE
DN 97378928 PubMed ID: 9234525
TI Immunization of mice with DNA encoding fragment C of tetanus toxin.
AU Anderson R; Gao X M; Papakonstantinopoulou A; Fairweather N; Roberts M; Dougan G
CS Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, UK.
SO VACCINE, (1997 Jun) 15 (8) 827-9.
Journal code: 8406899. ISSN: 0264-410X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199710
ED Entered STN: 19971105
Last Updated on STN: 19971105
Entered Medline: 19971023
AB Immunization of mice with Fragment C protein, the non-**toxic** C-terminal **domain** of **tetanus toxin**, will protect mice against lethal challenge with tetanus toxin. A plasmid, pcDNA3/tetC, which encodes a synthetic tetC gene expressed under the control of the human cytomegalovirus major intermediate early promoter/enhancer **region**, was constructed. Fragment C expression was observed in Chinese hamster ovary cells following transfection with pcDNA3/tetC. The immune response induced by intramuscular immunization with pure pcDNA3/tetC DNA was evaluated in a murine model. Anti-Fragment C serum immunoglobulin and proliferative responses in splenocytes were observed following two immunizations with pcDNA3/tetC. The major IgG subclass that recognized Fragment C was IgG2a and the stimulated splenocytes secreted high levels of interferon-gamma. Sufficient anti-Fragment C serum immunoglobulins were induced by DNA-mediated immunization to protect mice against lethal challenge with tetanus toxin.

L8 ANSWER 8 OF 8 MEDLINE DUPLICATE 5
AN 92340509 MEDLINE
DN 92340509 PubMed ID: 1634516
TI Minimal essential **domains** specifying **toxicity** of the light chains of **tetanus toxin** and botulinum neurotoxin type A.
AU Kurazono H; Mochida S; Binz T; Eisel U; Quanz M; Grebenstein O; Wernars K; Poulain B; Tauc L; Niemann H
CS Institute for Microbiology, Federal Research Center for Virus Diseases of Animals, Tubingen, Federal Republic of Germany.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Jul 25) 267 (21) 14721-9.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199208
ED Entered STN: 19920911
Last Updated on STN: 19970203
Entered Medline: 19920826
AB To define conserved domains within the light (L) chains of clostridial neurotoxins, we determined the sequence of botulinum neurotoxin type B (BoNT/B) and aligned it with those of tetanus toxin (TeTx) and BoNT/A, BoNT/C1, BoNT/D, and BoNT/E. The L chains of BoNT/B and TeTx share 51.6% identical amino acid residues whereas the degree of identity to other clostridial neurotoxins does not exceed 36.5%. Each of the L chains contains a conserved motif, HExxHxxH, characteristic for metalloproteases.

We then generated specific 5'- and 3'-deletion mutants of the L chain genes of TeTx and BoNT/A and tested the biological properties of the gene products by microinjection of the corresponding mRNAs into identified presynaptic cholinergic neurons of the buccal ganglia of Aplysia californica. Toxicity was determined by measurement of neurotransmitter release, as detected by depression of postsynaptic responses to presynaptic stimuli (Mochida, S., Poulain, B., Eisel, U., Binz, T., Kurazono, H., Niemann, H., and Tauc, L. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7844-7848). Our studies allow the following conclusions. 1) Residues Cys439 of TeTx and Cys430 of BoNT/A, both of which participate in the interchain disulfide bond, play no role in the toxification reaction. 2) Derivatives of TeTx that lacked either 8 amino- or 65 carboxyl-terminal residues are still toxic, whereas those lacking 10 amino- or 68 carboxyl-terminal residues are nontoxic. 3) For BoNT/A, toxicity could be demonstrated only in the presence of added nontoxic heavy (H) chain. A deletion of 8 amino-terminal or 32 carboxyl-terminal residues from the L chain had no effect on toxicity, whereas a removal of 10 amino-terminal or 57 carboxyl-terminal amino acids abolished toxicity. 4) The synergistic effect mediated by the H chain is linked to the carboxyl-terminal portion of the H chain, as demonstrated by injection of HC-specific mRNA into neurons containing the L chain. This finding suggests that the HC domain of the H chain becomes exposed to the cytosol during or after the putative translocation step of the L chain.

=>